Protein Analysis of Human RPE Cells Affected by Aging and Age-Related Macular Degeneration

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INTRODUCTION

Background: In the United States, Age-Related Macular Degeneration is the leading cause of blindness in people over the age of 65 (Friedman et al., 2004). More than 9 million Americans show signs of AMD, and the number of cases is projected to nearly double by 2050 (Cheung et al., 2013). A key to understanding AMD is examining the Retinal Pigment Epithelium (RPE), a single layer of cells that form a portion of the blood/retina barrier.

Previous work in our lab has shown that there are significant changes in protein content and increased mitochondrial DNA damage in RPE cells affected by AMD, suggesting that mitochondrial dysfunction plays a role in the pathology of the disease. Our hypothesis is that RPE cells from AMD donor eye samples will show protein expression consistent with mitochondrial dysfunction.

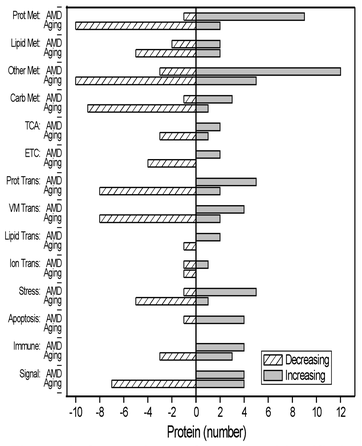
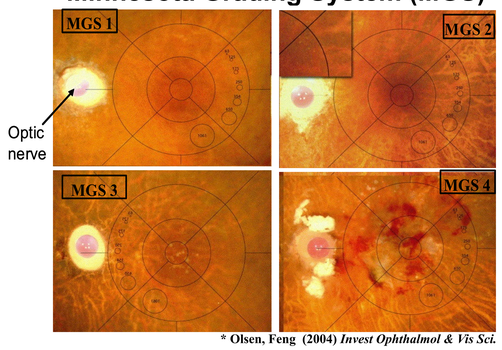


Figure 1. Comparison of proteins identified from the aging and AMD analyses graphed based on direction of change, from a previous study in the Ferrington lab.

In a previous analysis of human RPE cells, proteins were extracted and separated using 2-D gels. The spot intensities were analyzed and showed changes in protein content that were consistent with the hypothesis (Fig 1). The differences in protein content were also used to compare normal to pathologic aging. The purpose of this study was to confirm these results for select proteins using 1-D Western Blotting.

METHODS

Minnesota Grading System (MGS)*

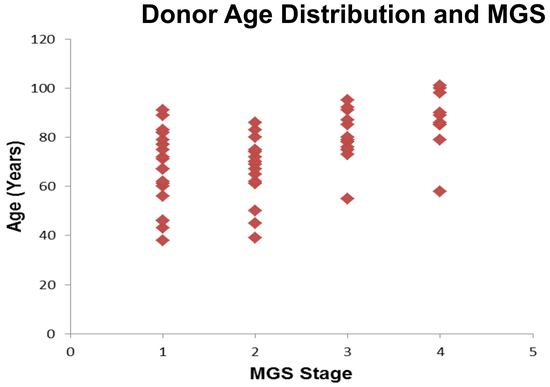


Donor Eyes Graded for The Stage of AMD

* Olsen, Feng (2004) *Invest Ophthalmol & Vis Sci.*

Image of human macula for donors without AMD (MGS1) and at progressive stages of AMD (MGS2 to 4). Dr. SANDRA MONTEZUMA graded the human donor eyes used in this study.

Donor Age Distribution and MGS Stage



MGS 1: 68 ± 3.4 (n=21)
MGS 2: 67 ± 3.0 (n=18)
MGS 3: 80 ± 2.7 (n=14)
MGS 4: 87 ± 3.6 (n=11)

METHODS

Experimental Approach

Human RPE cells (Graded MGS 1- 4)

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Extraction of cytosolic proteins

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Determine protein concentration by bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL, USA)

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Western Blotting to determine proteins present in each donor

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Image blots using ChemiDoc XRS (Bio-Rad) imager

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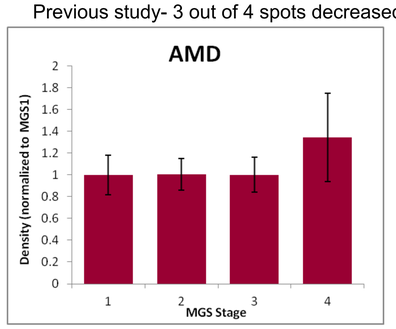
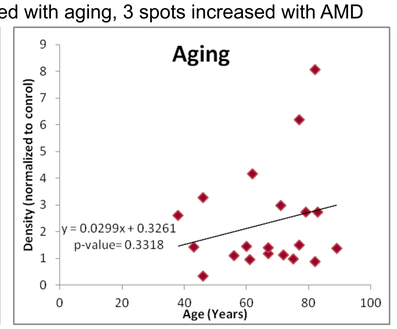
Measure density of each band using Quantity One software

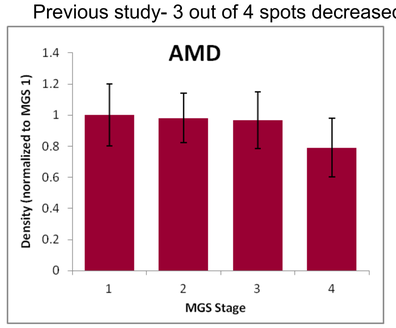
RESULTS

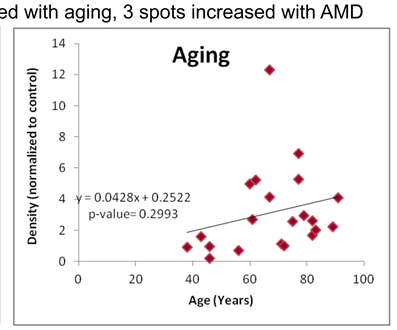
Cathepsin D (mature form): lysosomal protease
Previous study- 3 out of 4 spots decreased with aging, 3 spots increased with AMD



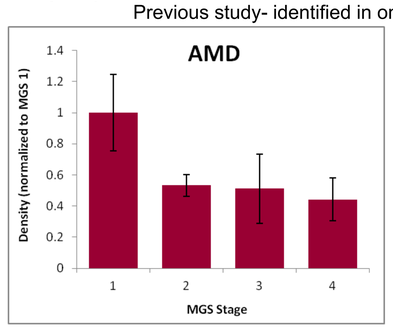


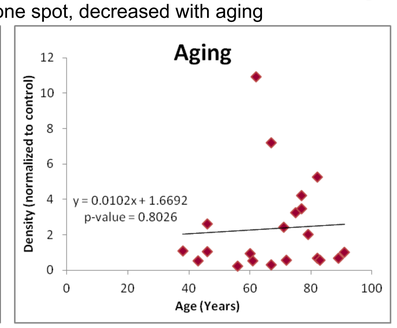
Cathepsin D (pre-/intermediate form): lysosomal protease
Previous study- 3 out of 4 spots decreased with aging, 3 spots increased with AMD





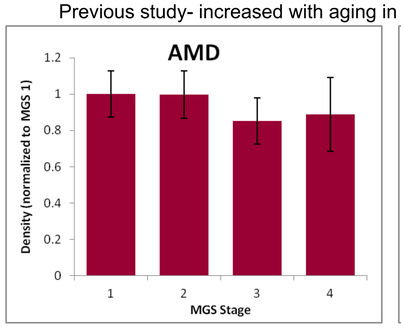
ERp29: protein of the endoplasmic reticulum, involved in protein transport and folding
Previous study- identified in one spot, decreased with aging

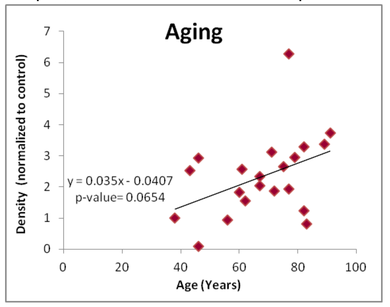




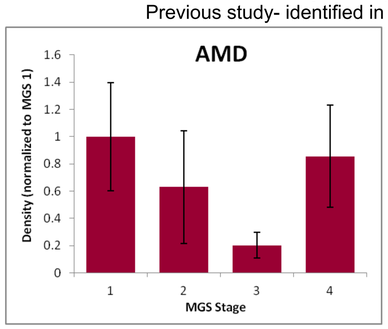
RESULTS

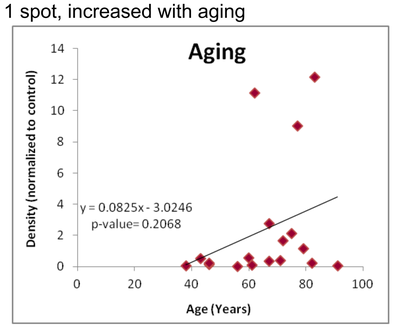
PDIA6: protein of the endoplasmic reticulum, involved in protein refolding
Previous study- increased with aging in 1 spot, increased with AMD in 1 spot



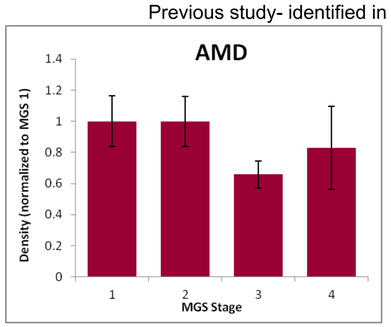


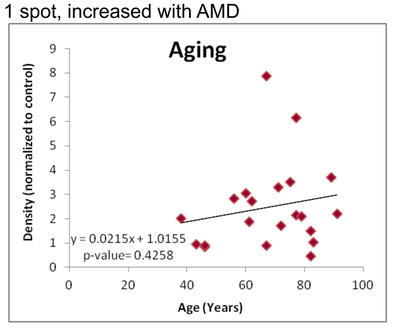
Transferrin: major transporter of iron in RPE cells
Previous study- identified in 1 spot, increased with aging





ERp46: Protein of the endoplasmic reticulum, involved in protein refolding
Previous study- identified in 1 spot, increased with AMD





CONCLUSION

- In our original study, PDIA6 increased with aging in a single spot; results from the current study show a trend towards increasing with aging.
- In the previous study, ERp29, ERp46, and Transferrin were identified in a single spot. Cathepsin D was identified in multiple spots.
- This study utilized 1D Western Blotting, whereas the original study used 2D gel electrophoresis and mass spectrometry. Because 2D gels separate proteins by size and charge, multiple isoelectric variants of the same protein can be detected in different spots, as the sensitivity of the mass spectrometry allows for multiple protein IDs per spot. The 1D gels utilized in this study will show the entire population of variants of the protein. This could explain some of the differences in trends between the two experiments.
- To continue this research, more samples are necessary to reduce error.

ACKNOWLEDGEMENTS

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